Ras proteins are membrane-associated, small guanine nucleotide-binding GTPases that control cell survival and proliferation. They consist of highly homologous catalytic domains and flexible C-terminal hypervariable regions (HVRs) that differ significantly across Ras isoforms, H-Ras, N-Ras, K-Ras4A and K-Ras4B, where *KRas4B* is among the frequently mutated oncogenes in human tumors. Recent NMR experiments discovered that the HVR of GDP-bound K-Ras4B extensively interacts with the catalytic domain. However, the HVR weakly interacts with the catalytic domain when the protein is in the GTP-bound state. Molecular dynamics (MD) simulations confirmed tight interaction of HVR with catalytic domain in the GDP-bound state, but not GTP-bound K-Ras4B, suggesting that in the GDP-bound state, an HVR domain could adopt a β-strand conformation, extending the β-sheet in the active site of the catalytic domain. Here we modeled K-Ras4B membrane interaction and dimerization. Membrane binding of K-Ras4B through the anchoring of the positively charged HVR is critical to its function as an oncogene and initiates signaling events. Recent studies showed that post-translationally modified HVR peptide spontaneously inserts the farnesyl group into the zwitterionic (DOPC) and anionic (DOPC:DOPS=4:1) bilayers in the liquid phase, but not into the DPPC bilayer in the gel phase. Further, spontaneous membrane insertion of the farnesyl group in full-length K-Ras4B strongly depends on the nucleotide type. The HVR of K-Ras4B-GTP preferentially interacts with lipids through the farnesyl insertion, while the HVR of K-Ras4B-GDP rather binds the catalytic domain and inserts less frequently the farnesyl into the lipid bilayer. Remarkably, K-Ras4B-GTP, but not GDP-bound, is able to form stable homodimers with different dimer interfaces, suggesting that the nucleotide-dependent dimerization with various dimer interfaces can resolve nanoclustering and cluster reorganization accomplishment with Raf's activation. Funded by Frederick National Laboratory for Cancer Research, National Institutes of Health, under contract HHSN261200800001E.

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Forming the Pseudomonas Aeruginosa Translocon Requires Simultaneous Incorporation of PopB and PopD

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Pseudomonas aeruginosa is a Gram-negative pathogen thatuses the Type III Secretion System to infect host cells. This system consists of a needle-like structure, by which proteins are secreted, and a translocon by which proteins are translocated across the plasma membrane of the target cell. The tip of the needle interacts with a hetero-oligomeric translocon formed by two proteins secreted through the needle: PopB and PopD. PopB and PopD can form pores individually or when mixed together. In model systems, mixed PopB and PopD assemble a unique hexadecameric complex containing 8:8 subunits of each protein. However, when not mixed, the translocators form mostly hexameric complexes in membranes. Does the formation of homo-oligomers interfere with the assembly of the hetero-oligomer?

While studying the insertion of the putative PopD transmembrane segment into the membrane we noticed that the location of the segment differed in the presence and in the absence of PopB. Formation of a heterocomplex positioned the PopD segment into a more hydrophobic environment. We reasoned that this conformational change could be employed to determine if the conformation adopted by PopD in homo-oligomers is affected by the subsequent addition of PopB. If reversible, formation of hetero-oligomers will change the location of the transmembrane segment to a more hydrophobic environment.

We found that the conformation adopted by PopD in homo-oligomers was not reverted by subsequent addition of PopB. These result is in good agreement with those showing that co-infection with two *P. aeruginosa* strains carrying only PopB and only PopD proteins are not able to translocate proteins into the target cells. Therefore, we conclude that an early interaction between PopB and PopD is required for the formation of a hetero-oligomeric translocon, and translocation of secreted effector proteins.

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Investigating the Functional Role of the Transmembrane Segments of Yta10, a Subunit of the mAAA Protease, in Membrane Protein Degradation

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The mAAA protease is present in the mitochondrial inner membrane and plays diverse roles in protein biogenesis and degradation. It is a hetero-oligomeric complex composed of Yta10 and Yta12. Yta10 carries two transmembrane segments (TMS) at its N-terminus and a large AAA+ domain and a proteolytic domain at its C-terminus. Previous studies have shown that the deletion of TMS does not compromise the proteolytic activity but, causes a defect in degradation of integral membrane proteins, hinting that the TMS of Yta10 is important for membrane protein degradation. The TMS of Yta10 has been either deleted or replaced by TMS of another mitochondrial inner membrane protein to elucidate their function. How Yta10 TMS mutants handle substrates have been tested using a set of Mgm1p of varying sequence composition in its 1st TMS. Mgm1p exists in two forms, l-Mgm1p and s-Mgm1p. The generation of s-Mgm1p is independent of the mAAA protease, but it becomes mAAA protease dependent when the first TMS of Mgm1p is replaced with a foreign TMS. A foreign TMS is dislocated from the membrane by the action of the mAAA protease and the downstream sequence is processed by Pcp1, generating s-Mgm1p. We speculated that if the TMS of Yta10 is required for the anchoring of the soluble domain to the membrane, only the deletion of TMS would impair the processing of Mgm1p variants to s-Mgm1p, not the substitution of TMS. In contrast, if the TMS required either for the recognition or dislocation of membrane proteins, both the deletion and substitution of TMS would impair the processing of Mgm1p variants to s-Mgm1p. This screening will elucidate the function of the TMS of Yta10 in membrane protein degradation.

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Oligomer Stoichiometry of Membrane-Bound Proteins Involved in a Cooperative Partition Equilibrium: A Homo-FRET Study

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An analytical framework that uses energy homo transfer to directly probe quantitatively the oligomerization state of membrane-bound proteins engaged in a three-state cooperative partition is presented [1]. It was assumed that monomeric proteins partition into the bilayer surface and reversibly assemble into oligomers with k subunits [2]. A general equation relating the overall steady-state fluorescence anisotropy of the sample to its fractional labeling was derived by considering explicitly that the anisotropy of mixed oligomers containing i-labeled monomers is inversely proportional to the number of labeled subunits per oligomer (Runnels and Scarlata limit). This method was very robust in describing the electrostatic interaction of Alexa 488 fluorescently-labeled lysozyme (Lz-A488) with phosphatidylserine-containing membranes. The pronounced decrease detected in the fluorescence anisotropy of Lz-A488 always correlated with the system reaching a high membrane surface density of the protein (low L/P molar ratio). The occurrence of energy homo transfer-induced fluorescence depolarization was further confirmed by measuring the anisotropy decays of Lz-A488 under these conditions. A global analysis of the steady-state anisotropy data obtained under a wide range of experimental conditions (variable anionic lipid content of the liposomes, L/P molar ratios and protein fractional labeling) confirmed that membrane-bound Lz-A488 assembled into oligomeric complexes, possibly with a stoichiometry of $k = 6 \pm 1$. This study illustrates that even in the presence of a coupled partition/oligomerization equilibria, steady-state anisotropy measurements can be used to monitor the self-assembly of membrane-bound proteins.

References

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